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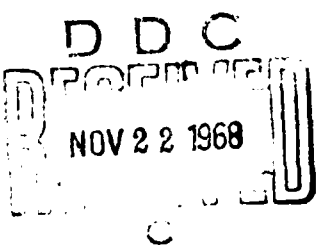
DATE: 24 October 1967



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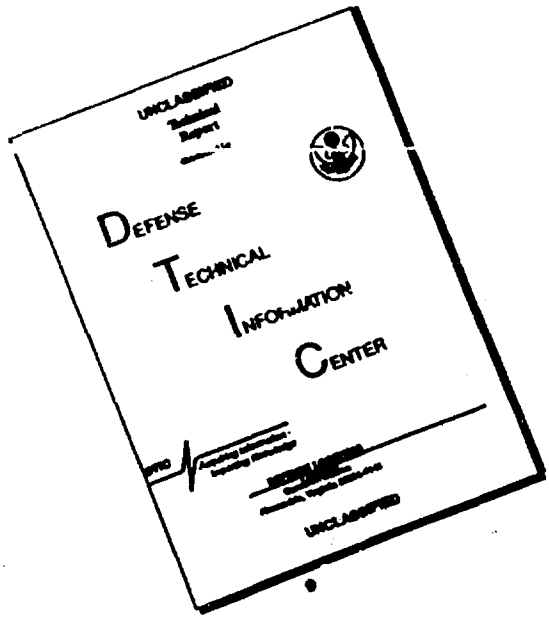

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UDC 576.858.13.095.383.095.6

MECHANISM OF THE EFFECT OF INTERFERON ON THE MULTIPLICATION OF VACCINIA VIRUS AND CELL METABOLISM

Voprosy Virusologii (Problems of Virology), Vol 11, No 6, 1966, pages 662-666.

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Many articles have been devoted to the study of interferon and its effect on the multiplication of various viruses. Articles on the study of the effect of interferon on the cell (1, 2, 4, 5) and cell metabolism under infection conditions have also been recently published.

In the present report are presented the results according to the study of the effect of interferon on the multiplication of the vaccinia virus and the metabolic processes occurring in the cells of chick fibroblasts and chorioallantoic membranes when they are inoculated with vaccinia virus.

MATERIALS AND METHODS

Vaccinia virus (dermovaccine strain) adapted to the cells of chick fibroblasts and to the chorioallantoic membrane of chick embryos was used in the experiments. A virus doge equal to 1000 ID_{50} for chick embryos and 1000 $TsPD_{50}$ /Expansion unknown/ for chick fibroblast cells was taken for inoculation.

Interferon is the allantoic fluid of chick embryos inoculated with duck influenza A, Koshitse strain 56. Elimination of the live virus was carried out by the acidification method; control in the absence of the virus was carried out by means of two successive passages on ten-day-old chick embryos. The antivirus activity of the indicated series was tested

*$TsPD_{50}$ Possibly TsPD [Tsvetnoy pokazatel' D_{50} ; Color Index D_{50}]

by the method of titration of 10,000-100,000 ID_{50} test virus on the chorioallantoic membranes, and against 100-1000 $TsPD_{50}$ in the case of titration on the tissue culture.

The interferon series obtained were active with respect to the test virus at dilutions of from 1:10 to 1:40, with a difference greater than 4 at 10 g_2.

An initially trypsinized tissue culture of chick fibroblasts incubated in a Lavrov culture medium with five percent bovine serum in test tubes with cover glasses was used in the experiments. Interferon was added to the two-day-old culture 18 hours before inoculation at a rate of 0.1 ml per one ml medium. Then the interferon was washed out and the cells were infected with vaccinia virus. After an hour's contact with the virus the tissue culture cells were washed clean of unadsorbed virus and Lavrov culture medium containing five percent bovine serum was poured over it.

In order to study the dynamics of vaccinia virus multiplication the untreated chick fibroblast tissue culture and the chick fibroblast tissue culture treated with interferon were investigated at various times after inoculation (after 1, 2, 3, 4, 5, 6, 8, 10, 24, 48, and 72 hours).

The amount of virus in the untreated cultures and those treated with interferon was determined in the hemagglutination reaction and by the method of biological titration on the chorioallantoic membranes of chick embryos and on chick fibroblast cells.

The synthesis of nucleic acids and proteins in the cells was studied by the autoradiography method. Thymidine-H^3 was used for studying nucleic acid metabolism and alanine-H^3 was used for the study of protein metabolism.

The radioactive isotopes were introduced into the tissue culture at the intervals mentioned above in the amount of 0.5 microcurie/ml (thymidine-H^3) and 1 microcurie/ml (alanine-H^3). After an hour's contact with the isotope the tissue culture cells were washed clean of unincorporated isotope three times with cold Hanks solution, fixed in a 1:3 mixture of acetic acid and alcohol, and dried. Then they were coated with MR emulsion. After four weeks exposure the preparations were treated with amidol developer, fixed, and stained by the Gimze-Romanovskiy method. The extent of incorporation of the radioactive isotopes in the cell was determined by means of the granule-autograph count per 100 cells (optical microscope).

Method of fractionation: Twelve-day-old chick embryos were treated with 0.5 ml interferon each on the chorioallantoic membrane 18 hours before inoculation. Embryos treated with interferon without subsequent inoculation with the virus served as the control.

Thirty minutes, 2, 3, 6 and 24 hours after inoculation with the vaccinia virus at a dilution of 10^{-2} in the amount of 0.2 ml per embryo the

chorioallantoic membranes of the test and control embryos (20 units in each group) were washed clean of erythrocytes with physiological solution, weighed, and carefully minced with scissors, and adenine tagged with radioactive carbon was added to each portion investigated, in the amount of 5 microcuries/ml. After an hour's contact with the isotope at 37° the cells were repeatedly washed with ice water to remove the radioactive isotope not incorporated in the cells. The washed cells were homogenised in porcelain mortars, twice that volume of 0.25 M sucrose was added, and fractionation was carried out according to the Schmidt-Thannhauser method (6), Davidson-Smellay modification. The free nucleotides were removed by 0.6 $HClO_4$ in the cold, and the nucleic acids were obtained by acid hydrolysis at 60°.

The radioactivity of the fractions obtained was determined in a volume of one ml, counted on the "Volna" apparatus with an end-window counter; the counting efficiency was 10 percent.

RESULTS

On studying the effect of interferon on vaccinia virus multiplication in the cells of a tissue culture of chick fibroblasts at various periods after inoculation it was shown that interferon appreciably suppressed the multiplication of vaccinia virus in these cells (Table 1).

TABLE 1

Effect of Interferon on Vaccinia Virus Multiplication

1) Время (в часах)	2) Клетки культуры ткани куриных фибробластов															
	3) обработанные интерферином								4) не обработанные интерферином							
	5) БОЕ				6) РГА				5) БОЕ				6) РГА			
7)	исход-ная	10^{-1}	10^{-2}	10^{-3}	7) исход-ная	10^{-1}	10^{-2}	10^{-3}	7) исход-ная	10^{-1}	10^{-2}	10^{-3}	7) исход-ная	10^{-1}	10^{-2}	10^{-3}
2	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
4	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−
6	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
8	−	−	−	−	−	−	−	−	+	+	+	+	++	+		
10	±	±	−	−	−	−	−	−	+	+	+		++	+++	+++	
24	±	±	±	−	+++	−	−	−	+	+	+	++−	+++	+++	+++	
48	±	±	±	−	+	++	−	−	+	+	+		+++	+++	+++	
74	+	+	+	+	++	++	−	−	+	+	+		+++	+++	+++	

Note: BOYe [blyashkoobrazuyushchiye yedinitsy ; patch-forming units on the chorioallantoic membrane of chick embryos] RGA [reaktsiya gemagglyutinatsii ; hemagglutination reaction data with a suspension of chorioallantoic membranes.]

Legend: 1) Time (in hours)
2) Cells of chick fibroblast tissue culture
3) Treated with interferon

[Legend continued on following page]

Legend (continued): 4) Not treated with interferon
5) Patch-forming units
6) Hemagglutination reaction
7) Initial

The autoradiography data on the study of nucleic acid metabolism showed that in the cells treated with interferon and infected with the virus no radioactive tracers were discovered in the cytoplasm in a single one of the observation periods. However, after 4-5 hours a small amount of the tracers appears in the nucleus, which is accounted for by the tagging of cell DNA. In cells not treated with interferon but infected with vaccinia, tracers are discovered in the cytoplasm four hours after inoculation (19 percent). After six hours the quantity of autographs increases to 26 percent and by 24 hours reaches 66 percent (Table 2), which indicates the synthesis of virus DNA.

TABLE 2

Incorporation of Radioactive Isotopes in Chick Fibroblast Cells

1) Время (в часах)	2) Тимидин-H^3								3) Аланин H^3							
	интерферон + заражение 5)		заражение 6)		4) контроль: интерферон 7)		4) контроль: % клеток 8)		интерферон + заражение 5)		заражение 6)		4) контроль: интерферон 7)		4) контроль: % клеток 8)	
	N	C	N	C	N	C	N	C	N	C	N	C	N	C	N	C
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	7	0	15	0	0	0	0	0	0	0	0	3
4	11	0	5	19	—	—	—	—	0	0	0	28	—	—	—	—
5	10	0	2	19	—	—	12	0	0	10	0	21	—	—	0	6
6	12	0	0	26	11	0	0	0	0	15	0	21	0	0	0	7
24	—	—	0	66	—	—	12	0	0	20	0	23	0	0	0	12

Note: N — nucleus; C — cytoplasm

Legend: 1) Time (in hours
2) Thymidine-H^3
3) Alanine-H^3
4) Control
5) Interferon + inoculation
6) Inoculation
7) Interferon
8) No. of cells

In the control cultures of cells treated with interferon alone no autographs are observed in the cytoplasm; they are found only in the nucleus (7-11 percent).

In cells not treated with interferon and not infected with the virus autographs also appeared only in the nucleus, but in larger amounts (12-15 percent; see Table 2).

On studying protein metabolism it was shown that treatment of the cells with interferon without subsequent inoculation suppressed the incorporation of alanine-H^3 in the protein fraction of the cells.

In cells treated with interferon and infected with vaccinia virus incorporation of alanine into the cytoplasm is somewhat lessened compared to the infected cells not treated with interferon.

The study of the effect of interferon on the synthesis of nucleic acids and nucleotides in the chorioallantoic membrane of chick embryos according to the capture rate of adenine-C^{14} in various fractions showed that 30 minutes after inoculation with the virus the incorporation of adenine into the DNA fraction of the cells which were not treated with interferon did not differ from that of the uninoculated control samples. Adenine-C^{14} capture in the DNA fraction of the cells treated with interferon and infected with the virus was twice as low as in the uninfected and virus-infected control cells.

The degree of incorporation of adenine-C^{14} in the nucleic acid fraction of the infected cells exceeded the degree of incorporation of the isotope in the controls by one-third (expressed in impulses per minute) (Fig. 1a).

In the cells treated with interferon without subsequent inoculation the incorporation of adenine in the DNA fraction, just as in the nucleic acid fraction, was almost indistinguishable from that of the controls (see Fig. 1a).

Three hours after infection the degree of incorporation of radioactive adenine into the DNA and nucleic acid fractions of the cells which were not treated with interferon and were infected with the virus was higher than in the control cells.

On treatment with interferon a drop in the incorporation of adenine-C^{14} was noted in all the fractions investigated (acid-soluble, nucleic acids and proteins).

Cells which were treated and infected with virus did not incorporate the radioactive isotope as intensively as the cells treated with interferon without inoculation. By six hours from the time of inoculation an increase was observed in the incorporation of adenine in the DNA fraction of the

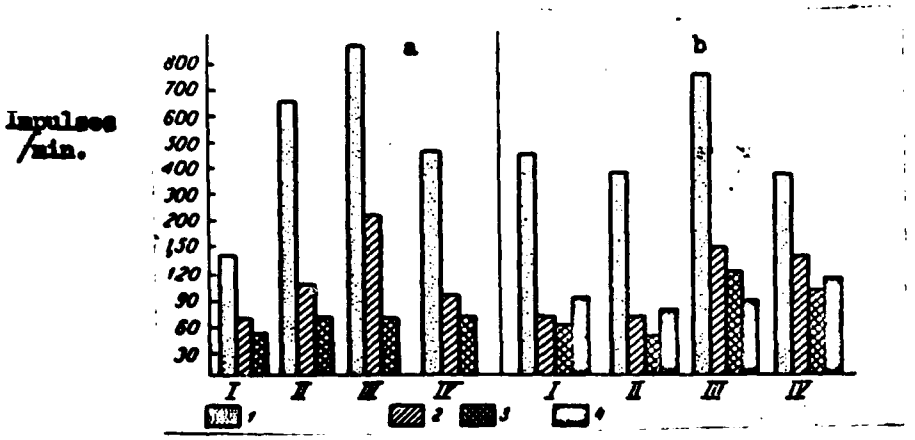

Fig. 1. Incorporation of Adenine-C^{14} in Various Fractions.

a — 30 minutes after inoculation; b — 5 hours after inoculation.
I — interferon and virus; II — interferon; III — virus;
IV — control. 1 — acid-soluble fraction; 2 — nucleic acid; 3 — DNA; 4 — protein.

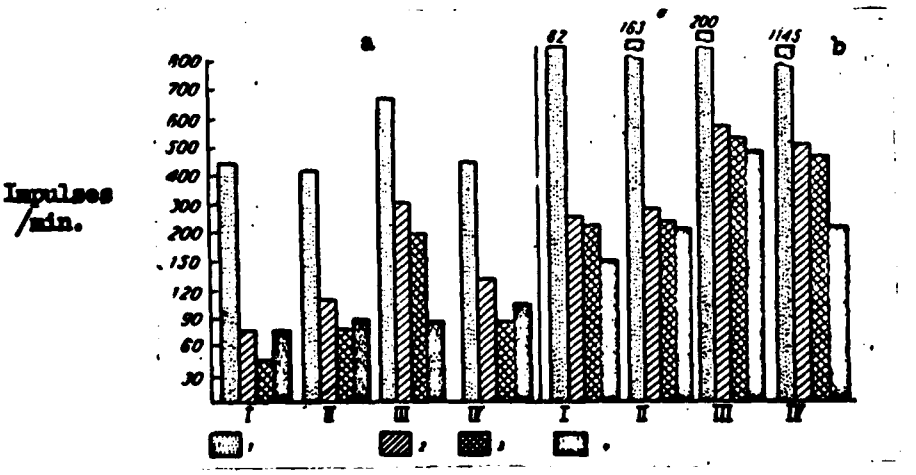

Fig. 2. Incorporation of Adenine-C^{14} in Various Fractions.

a — 6 hours after inoculation; b - 24 hours after inoculation.
The symbols are the same as in Fig. 1.

cells that were infected and not treated with interferon (Fig. 2a). Incorporation into the protein fraction did not differ from the controls.

Treatment with interferon reduced the adenine-C^{14} capture in all fractions (protein, nucleic acid, DNA) in the cells with subsequent infection and those without it.

Already by 24 hours after inoculation the incorporation of adenine-C^{14} in the DNA, protein, and nucleic acids of the untreated cells was higher than in the controls (Fig. 2b).

Upon treatment with interferon the adenine-C^{14} capture also decreased. In the group of cells treated with interferon and infected with virus it was even less active than in the preceding group.

The results of the investigations carried out by the autoradiography and fractionation method showed that interferon suppresses the multiplication of the vaccinia virus in chick fibroblast cells.

Preliminary treatment of the cells with interferon depresses the synthesis of virus DNA and to a lesser extent suppresses the synthesis of virus protein. In cells treated with interferon without subsequent infection the synthesis of DNA, and particularly of protein, is also suppressed. Infection intensifies the incorporation of the isotope into the DNA fraction three hours after inoculation, and into all the fractions studied after six hours. However, treatment with interferon before inoculation appreciably suppresses capture of the radioactive substance by the cells in all the fractions.

CONCLUSIONS

1. Interferon suppresses multiplication of the vaccinia virus in a chick fibroblast tissue culture.

2. Treatment of chick fibroblast cells with interferon before their infection with vaccinia virus suppresses the synthesis of virus DNA and, to a lesser extent, that of virus protein.

3. Interferon alters cell metabolism, suppressing the incorporation of adenine-C^{14} in all the fractions studied, and particularly in the protein fraction.

4. In the cells infected with vaccinia virus but not treated with interferon an increase in isotope capture is already noted in the DNA fraction after three hours, and in all the fractions mentioned above after 24 hours.

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Submitted to the editor
11 July 1965